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Abstract: Pyrogenic organic matter (PyOM) is considered an important soil carbon (C) sink. However, there are evidences that its addition to soil may induce a priming effect (PE) thus influencing its C abatement potential. The direction, the size and the mechanisms responsible for PyOM induced PE are far from being understood. We collected approximately 650 data points from 18 studies to analyse the characteristics of the PE induced by PyOM. The data- base was divided between the PE induced on the native soil organic matter and on fresh organic matter. Most of the studies were short-term incubation therefore the projections of findings on the long term may be critical. Our findings indicate that over 1 year PyOM induces an average positive PE of 0.3 mg C g ^{1} soil on native soil organic matter and a PE of approximately the same size but opposite direction on fresh organic matter. We studied the correlation of PE with several properties of soil, of the added PyOM, and time after PyOM addition. We found that PyOM primes positively the native soil organic matter in the first 20 days while negative PE appears in a later stage. Negative PE was correlated with the soil C content. PyOM characterized by a low C content induced a higher positive PE on native soil organic carbon. No correlation was found between the factors record in our database and the PE induced on the fresh organic matter. We reviewed the mechanisms proposed in literature to explain PE and discussed them based on findings from our meta-analysis. We believe that the presence of a labile fraction in PyOM may trigger the activity of soil microorganisms on the short term and therefore induce a positive PE, while on the long term PyOM may induce a negative PE by promoting physical protection mechanisms.

DOI: https://doi.org/10.1111/gcbb.12194

A meta-analysis on pyrogenic organic matter induced priming effect.

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Abstract

Pyrogenic organic matter (PyOM) is considered an important soil carbon (C) sink. However, there are evidences that its addition to soil may induce a priming effect (PE) thus influencing its C abatement potential. The direction, the size and the mechanisms responsible for PyOM induced PE are far from being understood. We collected approximately 650 data points from 18 studies to analyze the characteristics of the PE induced by PyOM. The database was divided between the PE induced on the native soil organic matter and on fresh organic matter. Most of the studies were short-term incubation therefore the projections of findings on the long term may be critical. Our findings indicate that over one year PyOM induces an average positive PE of 0.3 mg C g-1 soil on native soil organic matter and a PE of approximately the same size but opposite direction on fresh organic matter. We studied the correlation of PE with several properties of soil, of the added PyOM, and time after PyOM addition. We found that PyOM primes positively the native soil organic matter in the first 20 days while negative PE appears in a later stage. Negative PE was correlated to the soil C content. PyOM characterized by a low C content induced a higher positive PE on native soil organic carbon.

No correlation was found between the factors record in our database and the PE induced on the fresh organic matter.

We reviewed the mechanisms proposed in literature to explain PE and discussed them based on findings from our meta-analysis. We believe that the presence of a labile fraction in PyOM may trigger the activity of soil microorganisms on the short term and therefore induce a positive PE, while on the long term PyOM may induce a negative PE by promoting physical protection mechanisms.

Introduction:

Pyrolysis process consists in the heating of organic matter under anoxic conditions. Pyrolysis can occur during wildfires, where local and temporary limitation of oxygen can occur, or it can be a controlled process to produce heat and PyOM, also termed *biochar,* from agricultural residues (Lehmann & Joseph, 2009). We define here Pyrogenic organic matter (PyOM) as the residues of pyrolysis of biomass. PyOM can represent an important fraction of soil C both in soils amended with biochar and in natural soils. In fact PyOM is virtually present in every soil (Preston & Schmidt, 2006), and massive inputs of PyOM to the soil occur every year by forest burning. The estimations of PyOM global production vary between 40 to 600 Tg C $year^{-1}$ (Crutzen & Andreae, 1990; Kuhlbusch & Crutzen, 1995). Such a high input rate together with its centennial mean residence time (Singh *et al.*, 2012a) makes PyOM a fundamental component of soil organic matter. Moreover, PyOM content in soil is likely to increase in future due to the increasing fire frequency (Moritz *et al.*, 2012; Flannigan *et al.*, 2013) and to the growing interest in the use of PyOM - biochar - as a tool to increase soil fertility and store C at the same time (Lehmann, 2007; Verheijen *et al.*, 2010).

Many recent studies indicated that PyOM beside from being a stable C pool can also affect the stability of non-PyOM C pools, i.e. it may induce a priming effect (PE). Here we adopt the terminology from Bingeman *et al.*, (1953) who use the term PE to indicate the change in the mineralization rate of the soil organic matter in a soil receiving an input of exogenous organic matter. We specifically define PE as positive when the addition of PyOM increases the mineralization rate of non-PyOM C and negative when PyOM decreases the mineralization rate of non-PyOM C. PyOM was previously found to induce a positive PE (Wardle *et al.*, 2008; Luo *et al.*, 2011) or a negative PE (Kuzyakov *et al.*, 2009; Cross & Sohi, 2011). Also, no effect has been reported (Abiven & Andreoli, 2010; Santos *et al.*, 2012).

In the literature, PE was often reported as a response of soil to the addition of easily decomposable substrates like sugar (Wu *et al.*, 1993; Conde *et al.*, 2005; Blagodatskaya *et al.*, 2007, 2011; Blagodatsky *et al.*, 2010; Kuzyakov, 2010), cellulose (Fontaine & Barot, 2005) or plant material, e.g. rye grass (Wu *et al.*, 1993). Surprisingly it was found that also PyOM induced a priming effect although it has a low microbial availability (Kuzyakov *et al.*, 2009; Singh *et al.*, 2013). Several mechanisms have been proposed to explain the PE observed in individual studies; however a comprehensive analysis of results and mechanisms is still lacking.

Predicting the importance of PE induced by PyOM on the mineralization of non-PyOM C is crucial to assessment of PyOM C abatement potential. Woolf & Lehmann (2012), predicted the importance of PyOM induced PE on C stabilization in agroecosystem by using a process based model, however very little is known on the mechanisms responsible for PyOM induced PE. For example, it is still not known whether the PE is related to the type of soil and to the characteristics of the primed soil organic matter, or to the characteristics of the PyOM. It is also not known whether the PE is important on the long term, or if it is only a short-term phenomenon. The aim of the present study is to assess whether PyOM induces PE, its impact

on soil C budget, and which factors are driving its size and direction. To reach these goals we carried out a meta-analysis of the data reported in literature on PyOM induced PE separately on native soil organic matter and on fresh organic matter. We define here fresh organic matter, as an organic input added to the soil, e.g. plant material or glucose.

Moreover we reviewed the existing mechanisms presented in literature and discussed them based also on results from our meta-analysis.

Data collection

The PE induced by PyOM on the native organic matter and on the fresh organic matter was considered separately. We extracted data from individual sampling dates, in order to take into account the effect of time on PE. We expressed PE as the increase in CO_2 efflux derived from non-PyOM C pool compared to CO_2 efflux in the control treatment, i.e. in the treatment without PyOM addition. Where CO_2 efflux was not expressed as rate but as cumulative CO_2 efflux, we transformed the cumulative values into fluxes and attributed it to the last day of the cumulative period. To give the same importance to each study, we attributed to each data point a weight, inversely related to the number of data extracted from the study, using equation [1]:

$$W=1/N_{data} \qquad [1]$$

Where W is the weight attributed to individual data, and N_{data} is the number of data extracted from the study.

To estimate how soil respiration changed over time in treatments where no PyOM was added (i.e. in control treatments) we used a linear regression of soil respiration on log-transformed time (Figure 1 supplementary material). For PE on native soil organic matter we restricted our data collection to studies employing isotopic techniques to discriminate among the sources of

94 CO_2. In the case of studies using both labelled and unlabelled techniques (Zimmerman *et al.*,
95 2011) we considered only the treatments with ^{13}C-CO_2 estimations. We consider here that
96 only the ^{13}C-based studies can provide reliable information and direct calculation to the PE.
97 For the PE on native organic matter, we collected data from 12 publications (Table 1). Our
98 data set was composed in total of 464 data points, each of them reporting PyOM C content,
99 soil C content, time, soil pH, PyOM-C added g^{-1} soil C added, PyOM parent biomass (wood
100 or grass), soil texture (classes).

101 PE was calculated using equation 2:

102 $$PE = NSOM_{PyOM} - NSOM_{con} \quad [2]$$

103 Where PE = PE, $NSOM_{PyOM}$ = mineralization rate of Native Soil Organic Matter in soil
104 amended with PyOM, $NSOM_{con}$ = mineralization rate of Native Soil Organic Matter in control
105 (soil without any amendment). All the terms are expressed as mg C-CO_2 g^{-1} soil day^{-1}.

106 For the PE induced by the interaction between PyOM and fresh organic matter, we compiled a
107 data set from six studies (Table 2), consisting in a total of 198 data. We estimated PE using
108 equation 3:

109 $$PE = (MIN_{withPyOM} - MIN_{withoutPyOM}) / MIN_{withoutPyOM} \quad [3]$$

110 Where $MIN_{withPyOM}$ is the mineralization of the fresh organic matter in the PyOM treatment
111 and $MIN_{withoutPyOM}$ the mineralization in the control treatment.

112 In the studies where PyOM induced PE on fresh organic was measured in a soil matrix (i.e.
113 not in quartz sand like in Hamer *et al.*, (2004), it is necessary to partition the CO_2 between
114 three sources: PyOM, native soil organic matter, and fresh organic matter. This would imply
115 the use of two of different isotopic labels, i.e. ^{14}C and ^{13}C, as in the study from Blagodatskaya
116 *et al.*, (2011). Since such set up has not been used so far in studies on fluxes in PyOM
117 amended soils, we included also studies where isotopic techniques were not applied.

Therefore when it was not possible to separate the flux of fresh organic matter from the other flux we used equation 4:

$$PE = (SR_{observed}-SR_{theoretical})/SR_{theoretical} \quad [4]$$

Where $SR_{observed}$ was the SR in $SR_{(soil+fresh\_organic\_matter+PyOM)} - SR_{soil}$, and $SR_{theoretical}$ was the sum of $(SR_{fresh\_organic\_,matter+soil}-SR_{soil})+(SR_{PyOM+soil}-SR_{soil})$, therefore attributing the whole change in SR to the a change in the mineralization of the fresh substrate. We reported the PE as % of fresh organic matter decomposition rate. We also calculated the PE and expressed it as mg C g C-fresh organic matter added, using equation 5 and 6:

$$PE= (MIN_{withPyOM} - MIN_{withoutPyOM}) \quad [5]$$

$$PE = (SR_{observed}-SR_{theoretical}) \quad [6]$$

To compare PE on fresh organic matter with the PE on native soil organic matter, we then expressed it as mg $C\text{-}CO_2$ g^{-1} soil day^{-1}. We recognise, however that this value is affected by the quantity of fresh organic matter added to the soil in the different experiments.

We tested the following factors as explanatory variables for PE induced on fresh organic matter: time, fresh organic matter C:N ratio, PyOM-C, PyOM-N, PyOM-C:N ratio, fresh organic matter N content, fresh organic matter C, fresh organic matter addition rate and soil C.

To evaluate which factors are influencing PE, we used the model simplification approach described in Crawley (2007) consisting in seeking for the minimal adequate model. In order to estimate if the relation between the response variable (PE) and the explanatory variable was significant without presuming their distribution, we used the bootstrapping method with 1000 re-sampling iterations (Crawley, 2007) to establish whether the slope of the regression was significantly different from 0 ($p<0.05$). The regression was weighted by giving to each point

the weight in equation 1. All the statistical analyses were performed using the statistical software *"R"*. The software *"g3Data" (*http://www.frantz.fi/software/g3data.php*)* was used to extract data from the figures.

Results and discussion of the meta-analysis

The maximum positive PE on native soil organic matter mineralization was observed by Cross & Sohi, (2011) in an incubation experiment fifteen days after PyOM addition and was equal to 0.04 mg C-CO_2 g^{-1} soil day^{-1}, while the minimum PE (i.e. the maximum negative PE) observed was equal to -0.02 mg C-CO_2 g^{-1} soil day^{-1} after 90 days (Zimmerman *et al.*, 2011). The maximum observation period was the incubation experiment by (Singh *et al.*, 2012b), lasting for 5 years. Overall most of the data points collected in the data base were short-term measurements. In fact 50% of the data points we collected were measured within less than three months after PyOM addition to the soil (Figure 2 supplementary material). Overall the average weighted PE observed in literature was 0.0020±0.0003 mg C-CO_2 g soil day^{-1} ($p<0.001$, weighted t-test). Among the explanatory variables, time (logarithm transformed) and the interaction between time and PyOM-C content were significant ($p<0.001$, regression slope different from 0, boot strapping). We observed that PE decreased with time, and that PyOM having a low C content induced more positive PE on the short term (Figure 3 supplementary material). Most of the highest positive effect occurred within the first 20 days and with a low PyOM-C content, while most of the negative priming occurred on a longer time scale (Figure 2). Modelling PE over time, based on data collected in the database, we observed that the PE was positive until 200 days, and then negative (Figure 1). When PE was cumulated over time it was reaching a neutral PE, i.e the positive PE induced in the beginning was counterbalanced by the negative observed afterward, approx 600 days (Figure 1). We did not observe a correlation between PE and the other variables recorded in our database: PyOM

C content, soil C content, time, soil pH, PyOM-C added g^{-1} C added, PyOM parent biomass (wood or grass), soil texture (classes).

We calculated the integral of the curve relating PE and time, and we found that PyOM after one year of addition induced a cumulative positive PE equivalent to 0.3 mg C g^{-1} soil. This loss represented 15% (0.32 mg C–CO_2 g^{-1} $soil^{-1}$, Figure 1) of the average soil respiration in control treatment, i.e. where no PyOM was added (2.1 mg C–CO_2 g^{-1} $soil^{-1}$, Figure 1 supplementary). The theoretical influence of PE on the abatement potential of PyOM-C is reported in Figure 4 of the supplementary materials. Given that we did not find a correlation between PE and the amount of PyOM-C added to the soil, we considered a fixed PE of 0.3 mg C g^{-1}soil $year^{-1}$. The impact of PE on C abatement potential is inversely proportional to the amount of PyOM-C added g^{-1} soil. Therefore when low amounts of PyOM are added to the soil the impact of PE can be relevant. In the studies collected in our database the mode of the addition rate was about 10 mg PyOM-C g^{-1} soil (Figure 4 supplementary), in this case losses by PE would represent 3% of PyOM-C added. Such addition rate would correspond in an ideal soil having 1 g cm^{-3} and tilled down to 20 cm to an addition rate of 20 t PyOM-C $ha^{-1.}$ Considering a pyrolysis C yield of 37% (Woolf & Lehmann, 2012) approximately 54t of agricultural residues-C would be necessary to produce such amount of PyOM. This would correspond to the summation of the agricultural residues produced over several years. Therefore we believe that the quantity of PyOM added to the field would generally be lower than the mode of PyOM generally added in laboratory experiment and therefore PE may significantly reduce the abatement potential of PyOM.

In the data set on PE induced on fresh organic matter the longest observation period was 1.5 years in the incubation experiment by Liang *et al.*, (2010). The maximum observed PE was 53 % and was observed in Novak *et al.*, (2010) after 25 days, while the minimum was observed in Zavalloni *et al.*, (2011) where the decomposition of fresh organic matter plus PyOM did not

significantly differ from the decomposition of fresh organic matter alone. We found that the mean of PyOM induced PE on the fresh organic matter was negative and was -10±2% (weighted t-test, $p<0.001$) of the theoretical mineralization rate, this is equivalent to -3.9±1.5 mg C-CO_2 g C-fresh organic matter day^{-1} and to -0.005±0.002 mg C-CO_2 g^{-1} soil day^{-1} when expressed on a g of soil basis. We could not find a relation between recorded factors and PE. Nevertheless this could be attributed to the setups that differed considerably among the experiments, and this may have increased the variability of the response.

Woolf & Lehmann (2012) predicted using a process based model, that the cumulative PE induced by PyOM, at the end of the year, was negative. They assumed that the C stabilization effect of PyOM was linearly related to amount of PyOM content in soil, while we observed a correlation between PyOM-C and PE and no correlation with the application rate. If negative PE is considered to be positively correlated to PyOM content in soil, assuming a yearly input of PyOM to the soil, and given the low decomposition rate of PyOM, the C stabilization will increase year after year. Given our results we believe that assuming the C stabilization to be proportional to PyOM contained in soil, may lead to an overestimation of C stabilized particularly when predicting dynamics over decades. Moreover Woolf & Lehmann (2012) considered only the effect of PyOM on the fresh organic matter, for which however very few experiment exist (Table 2), and did not take into account the effect that PyOM can have on native soil organic matter which may counterbalance the impact on the fresh organic matter, as we observe from our results that the two effects have an opposite direction.

When looking at the PE induced by other types of organic matter, Sayer *et al.* (2011) found that litter input increased native soil organic mineralization by 13% over one year, while Crow *et al.* (2009) found that litter addition induced a priming of 15-21% over one year. This indicates that the PE induced by PyOM is equivalent to other types of organic inputs, despite its low decomposability.

Based on this meta-analysis, we conclude that both negative and positive PEs can co-exist. Looking at the native soil organic matter, the positive PE occurs on the short term and more intensively, while negative PE is acting on the longer term with less intensity. The characteristics of the PyOM (C content) also seem to play a role, but this is directly related to the time, i.e. PyOM characterised by a low C content may induce a positive PE on the short term. While for the PE on the fresh organic matter it was not possible to establish a relationship between the observed PE and the variables recorded in our database. Also, it has to be noticed that most of the studies included in the present meta-analysis are short-term (Figure 2 supplementary) and therefore projections of the impact of PE on the long term, may suffer from a lack of data. Moreover it has to be considered that on the long term the factors that may influence PE size and direction may vary, e.g. repeated addition of PyOM, seasonal variations of soil temperature and moisture. As these factors were constant in most of the studies considered (incubation studies) it was not possible to consider the effect of their variation over time.

In the literature, several mechanisms have been proposed to explain the PE induced by PyOM. In the following sections, we will review these mechanisms and evaluate how influential they can be considering also the meta-analysis results. We believe that the mechanisms hereafter described do not exclude each other and they may all contribute simultaneously to the resulting net PE.

The labile fraction mechanism

Our findings on the positive PE induced on native soil organic matter agree with the theory of Fontaine *et al.* (2003) and suggest that the addition of fresh organic carbon to the soil represents an energy source that increases the microbial biomass. According to the theory from Fontaine *et al.* (2003) if the added substrate is sufficiently complex this will favour the

239 growth of k-strategist decomposers responsible for the decomposition of the native organic
240 matter. In fact several studies observed an increase of soil microbial biomass after PyOM
241 addition (Bruun *et al.*, 2008; Steiner *et al.*, 2008a; Kolb *et al.*, 2009; Lehmann *et al.*, 2011;
242 Maestrini *et al.*, 2014a). Moreover PyOM, being a complex blend of molecules (Schmidt &
243 Noack, 2000; Keiluweit *et al.*, 2010; Spokas, 2010), is likely to represent the kind of substrate
244 that can trigger the growth of k-strategists microbes.

245 In addition, PyOM may contain a small easily decomposable fraction (Hamer *et al.*, 2004;
246 Keith *et al.*, 2011; Santos *et al.*, 2012; Singh *et al.*, 2012b; Maestrini *et al.*, 2014a) that would
247 constitute an energy source for microbial community on the short term. This agrees with our
248 findings that low-C content PyOM induce positive priming on the short term (Figure 1
249 supplementary materials). Several studies show that PyOM characterised by a low C content
250 contains a larger labile fraction. Singh *et al.*, (2012b), using ^{13}C cross polarization NMR
251 found that a low C content in PyOM was associated with a larger fraction of non aromatic C.
252 Fabbri *et al.*, (2012) found that a higher PyOM content in sugar, estimated by pyrolysis
253 coupled to gas-chromatography, was often associated to a low C content. Singh *et al.*, (2012b)
254 also found a positive correlation between soil respiration and the presence of an easily
255 decomposable fraction and Fabbri *et al.*, (2012) found that higher CO_2 fluxes were associated
256 to PyOM containing a higher fraction of sugars and a low level of aromaticity.

257 Even though we did not find a correlation between PyOM C content and pyrolysis
258 temperature there is a general agreement in the literature on the positive correlation between
259 the two (Keiluweit *et al.*, 2010; Zimmerman *et al.*, 2011). Also, the pyrolysis temperature is
260 likely to affect PyOM chemistry and thus its availability to soil microorganisms. Keiluweit *et*
261 *al.*, (2010) proposed a multi-phase model that correlates PyOM structure and pyrolysis
262 temperature. Their model proposes that the PyOM content of volatile matter (which is thought
263 to be more labile) decreases with higher pyrolysis temperature while the non-volatile fraction

(more resistant to decomposition) shows a relative increase. In this model a distinction is made between amorphous PyOM and composite PyOM. The amorphous PyOM is composed mainly of pyranones, phenols, pyrroles and is characterised by a relative increase of stable aromatic lignin compared to raw material, since lignin is more heat resistant than cellulose. The turbostratic PyOM is formed at higher temperature and is characterised by a higher degree of condensation. Turbostratic PyOM is composed of turbostratic crystallites embedded in a matrix of amorphous PyOM. It is therefore possible that less condensed and thus more available PyOM produced at low temperatures stimulates soil microorganisms inducing a higher positive priming effect.

Also the feedstock may affect PyOM chemical properties like PyOM-C content and forms. It is known that grass-derived PyOM are characterized by a lower C content and thus are less condensed, probably due to the lower thermal stability of cellulose compared to lignin (Hammes *et al.*, 2006; Keiluweit *et al.*, 2010; Knicker, 2010). For example, Hilscher *et al.*, (2009) using ^{13}C NMR technique, observed that a low C content of the ryegrass-derived PyOM corresponded to a higher content of alkyl C compared to the wood-derived PyOM. Overall we can therefore expect that grass-derived and low temperature PyOM induce a higher positive PE on the short term.

Sorption

This mechanism is related to the capacity of PyOM to adsorb other organic compounds. This property is already described extensively in the literature (Lehmann *et al.*, 2005; Lehmann & Joseph, 2009; Joseph *et al.*, 2010). PyOM capacity to adsorb organic compounds is related to its high porosity and cation exchange (Lehmann, 2007). It has been shown that PyOM has a higher sorption capacity than non-PyOM for dissolved organic matter (Cornelissen *et al.*, 2005a) and organic xenobiotics (Cornelissen *et al.*, 2005b).

Carbon stabilization by sorption is generally considered one of the most likely mechanisms for PyOM induced negative PE (Kimetu *et al.*, 2009; Kuzyakov *et al.*, 2009; Cross & Sohi, 2011), even though there is very little evidence for sorption of dissolved organic carbon to depress organic matter decomposition (Kaiser & Guggenberger, 2000). The PyOM sorption properties have been proposed as an explanation for negative PE from different perspectives. First, the PyOM has been hypothesized to adsorb part of the native soil organic matter, leading to a lower availability of substrate for decomposers (Kimetu *et al.*, 2009; Kuzyakov *et al.*, 2009; Cross & Sohi, 2011). Pietikäinen *et al.*, (2000) observed a high capacity of PyOM to adsorb dissolved organic carbon compared to pumice. Second, it has been proposed that PyOM sorption potential may influence enzyme activity. Zimmerman *et al.*, (2011) suggested that PyOM stabilization of extracellular enzymes alters their activity by: (i) blocking the active sites of the enzyme or (ii) by inducing a deformation on the tertiary structure of the enzyme. However these hypotheses were not confirmed by Jin, (2010) and Bailey *et al.*, (2011) who found an inconsistent effect of PyOM on a wide range of enzyme activities: the addition of PyOM to the soil in some cases increased enzyme activity, while decreased it in others. They attributed the increase in the activity to the PyOM stimulation of soil microorganisms and the decrease to the sorption of the assay on PyOM surface. Moreover, it has to be considered that among the two forms of enzymes active in the soil, the extracellular stabilized enzymes and the intracellular enzymes, only the latter contribute directly to microbial activity (Nannipieri & Gianfreda, 1998; Nannipieri *et al.*, 2012). The ecological role of extracellular stabilized enzymes is only indirectly connected to microbial activity as they may serve as a reservoir of potential enzymatic activity in case of changes in substrate availability and as originator of signalling molecules by cleaving off small fragments of larger polymer. The signalling molecules act as an inducer for microbes to release the enzyme for the target molecules (Gianfreda & Rao, 2011; Wallenstein & Burns, 2011). Therefore we believe that the sorption of extracellular enzymes on clays and organic matter (including

PyOM) can not decrease the microbial activity, although it can decrease the enzyme activity measured using the classical approach which can not separate between extracellular enzymes and enzymes associated to microbes (Nannipieri *et al.*, 2012).

In our meta-analysis the size of the negative PE was higher when the soil had a higher content in C when data on negative PE are pooled together (Figure 3). The sorption of dissolved organic carbon agrees with these findings: in fact the dissolved organic carbon production is not directly related to the time, and the physical protection mechanism is likely to become relatively more influential in a second stage when the positive PE has ceased. Also, the dissolved organic carbon production is proportional to the amount of C in the soil (Kalbitz *et al.*, 2000). This confirms findings from Zimmerman *et al.*, (2011), and Stewart *et al.*, (2013) who observed a higher C stabilization in soil richer in PyOM over a two years incubation. Zimmerman *et al.*, (2011) suggests that soil more rich in C will produce more C stabilization due to the higher potential C to be sorbed. However, Kuzyakov *et al.*, (2009) observed C stabilization in a loess sediment (very poor in C) but not in a C richer loess derived-soil, which although it was generally poor in C was still ten times richer in C than the loess sediment. Also, Chen *et al.*, (2008) showed that while non-PyOM sorption capacity is linearly dependent on the concentration of the solute the PyOM sorption capacity is non-linearly dependent, and shows greater affinity at low concentration rates, challenging the theory that negative PE is correlated with DOC content of soil. Nevertheless, the range of concentrations where PyOM sorption capacity is not linear is lower than the typical concentration of dissolved organic carbon in soil (mg C L^{-1}), therefore in the soil the sorption capacity of PyOM is likely to be linearly correlated in the concentration range of dissolved organic carbon.

Chun *et al.*, (2004) and Chen *et al.*, (2008) found that the pyrolysis temperature significantly altered the sorptive capacity of PyOM. They found that pyrolysis temperature altered the ratio

339 of carbonized/non-carbonized material that are characterised by different adsorption
340 properties. Also James *et al.*, (2005), Bornemann *et al.*, (2007) and Harvey *et al.*, (2011)
341 found similar results with increasing sorption affinity positively correlated to pyrolysis
342 temperature. It is therefore possible that charcoal produced at higher temperature has higher
343 sorption capacity. This would strengthen the importance of PyOM-C content which maybe
344 correlated on the one side to the size of the PyOM labile C content and on the other for PyOM
345 sorption capacity. Therefore high temperature PyOM may contribute to stabilize C in the soil,
346 while low temperature C may contribute to stimulate microbial activity.

347 Overall the sorption of dissolved organic matter on PyOM surface is a likely explanation for
348 the negative PE, particularly it is possible that in concurrence with the labile fraction it
349 determines the temporal pattern of PE of initial positive PE followed by a phase of negative
350 PE, as observed in several studies (Singh *et al.*, 2012b; Maestrini *et al.*, 2014a).

351 ## Mechanisms related to the changes of soil properties

352 Addition of PyOM to the soil may change the soil chemical and physical properties. There is
353 an increasing body of evidence that PyOM addition will increase soil pH, mostly by adding
354 ash to the soil (Van Zwieten *et al.*, 2009; Liu & Zhang, 2012; Maestrini *et al.*, 2014a). Luo et
355 al., (2011) observed a relatively higher initial PE in a soil with a lower pH, however we did
356 not find a relation between priming and soil or PyOM pH (data not reported). Nevertheless
357 the alteration of pH may affect organic matter mineralization in several ways. When the pH of
358 soil solution is higher than 5 this may alter the equilibrium between CO_2 and carbonates
359 (HCO_3^-, and CO_3^-) promoting the formation of the latter. This results in an artificial lowering
360 of the CO_2 efflux until the saturation of the soil solution is reached, this generally occurs
361 within a few days (Blagodatskaya & Kuzyakov, 2008; Lehmann *et al.*, 2011). Theoretically
362 the relevance of this process decreases with time, as PyOM amendment effect on pH has been

363 shown to decrease (Cheng *et al.*, 2006; Maestrini *et al.*, 2014a). However we observed that
364 most of the negative PE occurred in later stages, therefore we believe that this is not a main
365 driver for observed negative PE.

366 A shift in soil pH by PyOM addition may affect enzymatic activity (Jones *et al.*, 2011). Even
367 though we cannot rule out this hypothesis, each enzyme has its optimum and it is therefore
368 likely that a shift in pH does not have a unidirectional effect on enzyme activity.

369 Kimetu & Lehmann, (2010) suggested that PyOM may increase soil aggregation which can in
370 turn increase C stabilization, particularly by incorporating C in microaggregates (Six *et al.*,
371 2006). However, it is often not possible to distinguish the effect of sorption from the effect of
372 aggregation, in fact these two effects are often classified together as physical protection
373 mechanisms (Sollins *et al.*, 1996). Vasilyeva *et al.*, (2011), Brodowski *et al.*, (2006) and
374 Skjemstad *et al.*, (1993), found relatively large quantities of PyOM in microaggregates, and
375 propose that PyOM plays an important role in microaggregates formation acting as a binding
376 agent. A similar conclusion was drawn by Fellet *et al.*, (2011) who observed a higher water
377 retention capacity in PyOM amended soil and attributes this to an increase of aggregation.
378 Piccolo *et al.*, (1997), found that coal derived humic substances can improve aggregate
379 stability by creating a water repellent coating to the humic-mineral associations. Liang *et al.*,
380 (2010) found that PyOM increased the incorporation of fresh added organic matter in the
381 aggregates. However in their study this was not accompanied by a decrease of the PE induced
382 by PyOM on the fresh organic matter. We would expect that the promotion of aggregates
383 formation would have pronounced effects in soil with little structure, like sandy soil, however
384 we did not find an effect of soil texture on PE (data not reported), and therefore although we
385 believe that this mechanism can occur we can not confirm this with our meta-analysis.

386 An alternative explanation for the phase of negative PE observed in several studies is the
387 shortage of available organic matter caused by the higher decomposition rates of the positive

PE phase (Bingeman *et al.*, 1953). However, Hamer & Marschner, (2005) have shown by repeated substrate addition that there was no limitation in availability of soil organic carbon to PE. Moreover when this mechanism occurs we expect that a soil richer in C would be less affected by C depletion effect, but results on negative PE did show that soils richer in C are affected by more negative PE.

Mechanisms related to the change in microbial community and activity

The shift in the microbial structure is an explanation that has been used to explain both positive and negative PE. Zimmerman *et al.*, (2011) proposes that compounds toxic to microbes can be released from the PyOM and reduce microbial activity and organic matter mineralization. However they reject this hypothesis since the toxicity should show its effect on microbial biomass on the short term, due to the volatile fraction they contain. Our results on the PE occurring mostly on the short term, confirm that even if such mechanism exists it is not prevalent at least on the short term. Moreover there is also an increasing body of evidence reporting an increase in microbial biomass following PyOM addition that indirectly do not confirm the presence of toxic compounds (Bruun *et al.*, 2008; Steiner *et al.*, 2008b; Kolb *et al.*, 2009; Schneider *et al.*, 2011; Maestrini *et al.*, 2014a). Nevertheless, it is also possible that the observed increase in microbial biomass derives from an increased turnover following from the death of microbial groups sensitive to the toxic compounds. In this case the dead microbial cells could represent a growing substrate for microbes less sensitive to the toxic elements of PyOM, generating an increased turnover of microbial biomass (Blagodatsky *et al.*, 2010).

Lehmann *et al.*, (2011) proposed based on the observation that PyOM induces a negative PE and an increase in microbial biomass that the PyOM capacity of adsorbing organic matter

412 increases microbial efficiency (i.e. microbes produce less CO_2 for the same amount of C
413 incorporated). They suggested that microbial biomass increase may be due to co-location of
414 micro-organisms and soil organic matter; this would reduce the energy invested by microbes
415 in producing extracellular enzymes. The basis for this hypothesis is the *autoinducer* theory
416 (Redfield, 2002) that postulates that microbes release the enzymes only when the risk of
417 losing them by diffusion is minimal. According to such theory a concentration of
418 decomposable substrate higher than the quorum sensing, i.e. the substrate concentration
419 threshold for bacteria to produce extracellular enzymes, will induce the production of
420 extracellular enzymes, and therefore increase substrate decomposition and minimize the
421 expenses for the production of enzymes.

422 Our database does not allow us to verify the hypotheses on the efficiency of microbial
423 biomass in PyOM amended soils, however our results on positive PE induced by PyOM on
424 the short term are challenging the initial assumption from Lehmann *et al.*, (2011). Also Jones
425 *et al.*, (2012), who estimated microbial efficiency based on initial consumption rate of added
426 glucose found that microbial efficiency in PyOM amended soil decreased, therefore not
427 confirming this hypotheses.

428 Wardle *et al.*, (2008) observed in a litter bag experiment an increase in C mineralization from
429 a mix of charcoal and humus compared to the expected decomposition from the two
430 substrates separately. They suggested that PyOM can act as a *"foci"* (Zackrisson *et al.*, 1996),
431 i.e. a spot, where microbes can grow and decompose the phenols sorbed on PyOM surface
432 being protected from microarthropod predation. In fact according to Warnock *et al.*, (2007)
433 and Zackrisson *et al.*, (1996) the size of many charcoal pores (often below 16 µm in
434 diameters) would allow the entrance of bacteria, fungi and microbe-feeding nematodes but not
435 of predators like protists (8-100 µm) nor micro-arthropods (100µm-2mm). However, as
436 Lehmann *et al.*, (2011) recently reviewed there is no quantitative evidence that microbes

437 inside the PyOM porosity are protected from predators. Moreover the theory that microbial
438 activity is enhanced by protection against predation is challenged by the many evidences that
439 predation of microbes increase CO_2 efflux for example by increasing the activity of soil
440 microorganisms by transporting them to unexploited substrates and by providing excretion
441 and defecations that are readily usable (Ingham *et al.*, 1985).

442 ## Mechanisms for priming effect on fresh organic matter

443 The mechanisms proposed in literature for PE on fresh organic matter do not differ
444 substantially from the one proposed for the native soil organic matter. Wardle *et al.*, (2008)
445 suggests the foci hypotheses, similarly Hamer *et al.*, (2004) proposed that PyOM offers a
446 large surface for the growth of microorganisms. Zavalloni *et al.*, (2011) observed a
447 stabilization effect mixing PyOM and wheat straw, and suggested that the addition of PyOM
448 and decomposable organic matter produces an increase of C immobilization in microbial
449 biomass. However incubations are generally short term, and therefore the possible
450 immobilization of C in microbial biomass would be released on the long term by microbial
451 turnover. Jones *et al.*, (2012) suggests that PyOM induces a shift in the microbial community
452 promoting less C-efficient microorganisms, so that more C is respired and less is stored. Keith
453 *et al.*, (2011) report that PyOM may stabilize fresh organic matter by trapping it into organo-
454 mineral fractions. This was confirmed by Liang *et al.*, (2010) who found that more fresh
455 organic matter was incorporated in the aggregate fraction in a soil containing high quantities
456 of aged PyOM.

457 Looking at all the different reported explanation it appears clear that the labile theory does not
458 apply for the fresh organic matter, in agreement with the principle that fresh organic matter is
459 generally a source of energy for microbes, while old soil organic matter is rather a source of
460 nutrients (Fontaine *et al.*, 2003). On the other hand soil organic matter is rich in soluble
461 compounds that once in soil can be mobilised and trapped in the aggregates whose formation

462 is promoted by the presence of PyOM. Although the limited number of studies and the
463 heterogeneity of their setup does not allow us to conclude on the definitive mechanisms
464 responsible for the often reported PE on the fresh organic matter, we believe that it is likely
465 that PyOM may stabilize fresh organic matter by physical protection mechanisms.

466 Research perspectives

467 Although much work has been done on the mechanisms responsible for the priming effect, a
468 big uncertainty still resides in the processes occurring at landscape levels that may influence
469 PE induced by PyOM. We believe that particularly three aspects has to be elucidated: (i)
470 impact of continuous input of organic matter, (ii) impact of different land uses. Very little is
471 known on the impact of continuous input of fresh organic matter, their impact can be partially
472 inferred from studies where fresh organic was repeatedly added to the soil and biochar mix
473 (Hamer et al., 2004, Kuzyakov et al., 2009). Nonetheless, repeated input of the same
474 substance or mix of substances can hardly reproduce of the diversity and continuity of
475 rhyzodeposition.

476 Land use is a relevant driver for organic matter dynamics in soils, for example compaction, or
477 litter production seasonality strongly influence decomposition. Nonetheless so far studies
478 investigating PyOM induced PE only looked at the influence of different land uses on soil
479 organic matter quality and quantity (Cross et al. 2011). We believe that understanding the
480 impact of tillage on priming effect induced by PyOM is specially important in the context of
481 biochar application. However this aspect was never investigated in the field, although its
482 impact was simulated in incubation experiments by mixing of soil (Kuzyakov et al., 2009).

483 We believe that these two aspects are crucial to understand the impact of PyOM induced
484 priming effect on soil C budget.

Conclusions

We found that on average PyOM induces a priming effect of similar magnitude but opposite direction on native soil organic matter (positive priming effect) and fresh organic matter (negative priming effect). The priming effect on native soil organic matter was found to be related with time and PyOM-C content, with the positive priming effect occurring mostly on the short term and induced by PyOM characterized by a low C content, and negative PE appearing at a second stage.

We discussed the different mechanisms that can be involved in the PyOM induced priming effect on the native soil organic matter. We believe that the presence of a labile fraction in PyOM may induce a positive priming effect on the short term by triggering the activity of soil microorganisms. Simultaneously PyOM may promote the physical protection of organic matter by sorption on PyOM surfaces or into microaggregates, however the effect of this mechanism appears only in a second stage when positive priming effect has ceased.

We conclude that, although many uncertainties still exist, particularly on the parameters driving the amplitude and the direction of priming effect, adding PyOM to the soil induces a cumulative positive priming effect on a yearly time scale, on the native soil organic matter, which may be counterbalanced by the negative priming effect observed on fresh organic matter. However, further investigations on the factors influencing the priming effect induced on fresh organic matter are required; particularly studies employing double isotopic labelling would allow the determination of the priming effect both on the fresh and on the native soil organic matter.

Acknowledgments

We are grateful to three anonymous reviewers who carefully read the manuscript and provided insightful comments.

511 # Tables

512 Table 1: Observations of priming on the native soil organic matter, for the study without isotopes the PE column reflect whether PyOM addition
513 induced changes in soil respiration rather than in native soil organic matter mineralization. Only studies employing isotopes were then included in
514 the meta-analysis.

Author	**Type of experiment**	**Soil type**	**Biochar type**	**Climate**	**PE direction**	**Mechanism proposed**
(Bell & Worrall, 2011)	Lysimeter in the field without isotope	1)arable soil (Sandy clay loam), vegetated and unvegetated 2)Black humified peat/organic	Lump wood (C content approx. 80%)	Temperate	Mostly **Neutral** in the unvegetated treatment **Positive** in 2 out of 9 treatments.	Short-term increase of microbial biomass due to increased habitability (foci hypotheses).

(Cheng *et al.*, 2008)	Incubation of soil sampled next to a furnace with an adjacent, no isotopes (unknown quantity of PyOM in the soil)	Broad variety of Canadian soils	Charcoal was produced in furnaces	Gradient of soils	**Negative**	
(Cross & Sohi, 2011)	Incubation, with isotopes	Silty-clay loam 1)bare (C content 1%) 2)Arable C content(1.9%) 3)grassland (C content 3.6 %)	Range of sugar bagasse with different charring temperature (350-550 °C) and time (20-80)	Temperate	**Neutral** or **negative** when calculated on a C basis	Sorption, particularly of readily available substrates

(Jones *et al.*, 2011)	Incubation with isotopes	Cambisol	Mix of wood	Temperate	**Negative**	Several hypotheses, among them: 1)Sorption, 2)Enzymes sorption 3) liming effect
(Keith *et al.*, 2011)	Incubation with isotopes	Clay, rich in smectite, Vertisol	Eucalyptus Salinga	Queensland Australia	**Positive**, particularly in the first 18 days for SOM	Labile content of PyOM promote mineralization of native soil organic matter,ko
(Kolb *et al.*, 2009)	Incubation no isotopes	Several types	Mix of manure and pine wood	4 different soils series	**Positive**	Increase in microbial biomass responsible for the PE

						(foci). The strongest PE comes from the more fertile soils
(Kuzyakov *et al.*, 2009)	Incubation using stable isotopes	Loess soil and Loess	Grass L. perenne (400°C)	Temperate	**Neutral** (in loess derived soil) or **negative** (in Loess)	In Loess sorption of nutrients and organic C
(Luo *et al.*, 2011)	Incubation with isotopes	Aquic Paleudalf (Silty loam)	Miscantus giganteus	Temperate	**Positive** PE, relatively higher in the low pH soil, higher from PyOM of	Co-metabolism, due to the high concentration of available (i.e. dissolved) C

					350 °C	present in biochar. Moreover the 350 induced more priming than the 700. Relative higher PE in low pH than in high pH (liming induced PE).
(Maestrini *et al.*, 2014a)	Incubation with isotopes	Cambisol	Rye grass	Temperate	**Positive** in the first 18 days **negative** from day 18 to day 158	Co-metabolism and sorption of organic matter.

(Major *et al.*, 2010)	Field experiment with plants, with isotopes	Savanna Oxisol	Mangifera Indica (500 °C)	Tropical	**Positive**	PyOM induced more plant growth resulting in higher autotrphic respiration.
(Novak *et al.*, 2010)	Incubation without isotopes	Loamy sandy	Shell pecan	Subtropical	**Neutral**	
(Santos *et al.*, 2012)	Incubation with isotopes	Sandy loam and salty silt loam	Pinus ponderosa	Mediterranean	**Neutral**	
(Zimmerman *et al.*, 2011)	Incubation with isotopes	Several soil types	Grass and wood	Subtropical	**Positive** in the first 90 days and **negative** afterward	Co-metabolism in the beginning and encapsulation

						and/or organic matter sorption ob biochar surface.
(Singh *et al.*, 2012b)	Incubation with isotopes	Clay	Several (wood/leaf/manure, 400-550°C, under N_2, activated or not activated).	Queensland (Australia)	**Positive** in the first two years, and **negative** afterward.	
(Farrell *et al.*, 2013)	Incubation with isotopes	Coarse texture	Wheat and Eucalipt shoots	Temperate Mediterranean	**Positive**	Co-metabolism
(Kimetu *et al.*, 2009)	Incubation without isotopes	Clay	Wood (450°C) eucalyptus salinga	Tropical	**Negative** in the low C soil and no difference in soil	PyOM sorbs dissolved organic C or PyOM promotes

					respiration in the high C soil.	aggregation

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517 Table 2: Studies investigating the PE on the fresh organic matter. The column entitled “Formula for PE” indicates whether equation 4 or equation 3
518 was used to measure PE. Equation 4 was used when it was not possible to discriminate between the CO_2 derived from PyOM and the CO_2 derived
519 from fresh organic matter.

Author	**Type of experiment**	**Soil type**	**PyOM characteristics type**	**Co-substrate**	**Climate**	**Formula for PE**	**Mechanism proposed**	**PE direction**
(Abiven & Andreoli, 2010)	Incubation without isotopes	Cambisol with 30% clay, 4% organic C and pH 6.1	Picea abies, 450 °C 5 h anoxic	Four different co-substrates with a range of material	Temperate	Equation 4		**Neutral**
(Hamer *et al.*, 2004)	Incubation with isotopes	Quartz sand	Maize, Rice, Straw	Glucose	Temperate	Equation 3	Co-metabolism	**Positive**
(Zavalloni *et al.*, 2011)	Incubation without isotopes	Silt-loam Cambisol	Wood	Wheat-straw	Temperate	Equation 4		**Negative**
(Jones *et*	Incubation with	Sandy clay	Wood	Rye grass and	Temperate	Equation 3		**Negative** for

al., 2012)	isotopes	loam		glucose, aminoacids cocktail				rye grass, **neutral** for other substrate
(Keith *et al.*, 2011)	Incubation with isotopes	Clay, Vertisol	Eucalyptus Salinga	Sugar Cane residue	Subtropical	Equation 3	PyOM promotes incorporation of fresh organic matter in aggregates	**Negative**
(Liang *et al.*, 2010)	Incubation with isotopes. Terra preta (soils containing old PyOM) contrasted to adjacent soil (poor in PyOM)	Anthrosol	Already present in the soil		Tropical	Equation 3	Fresh organic matter incorporation in the Anthrosol rich in PyOM was higher than in the adjacent soil (poor in PyOM), but this did not result in a decrease of mineralization.	**Neutral**.
(Wardle *et al.*, 2008)	Litter bag, without isotopes		Empetrum hermaphroditum	Forest humus	Boreal	Not used	The “foci hypotheses”	**Positive**

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783 246.
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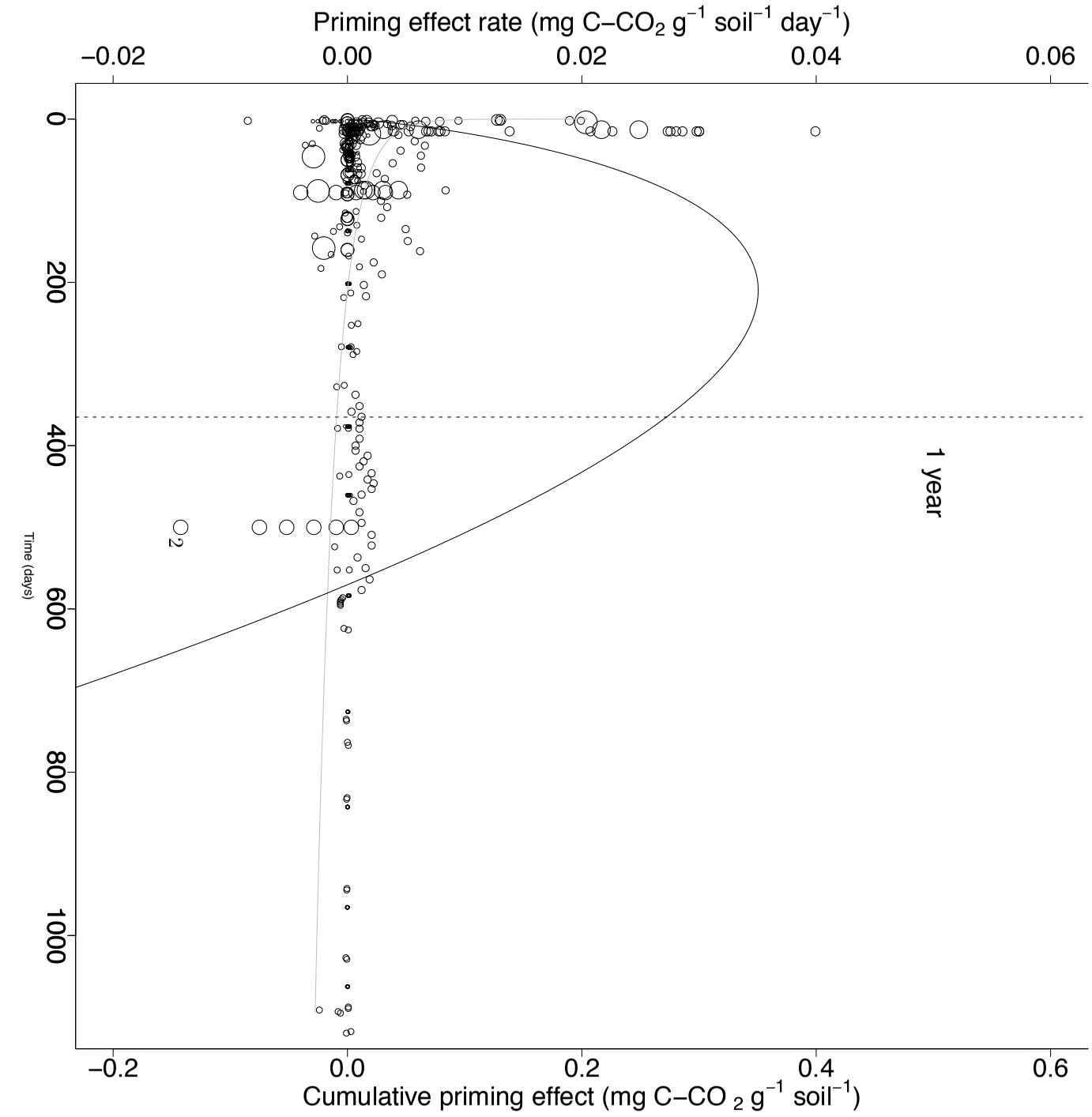
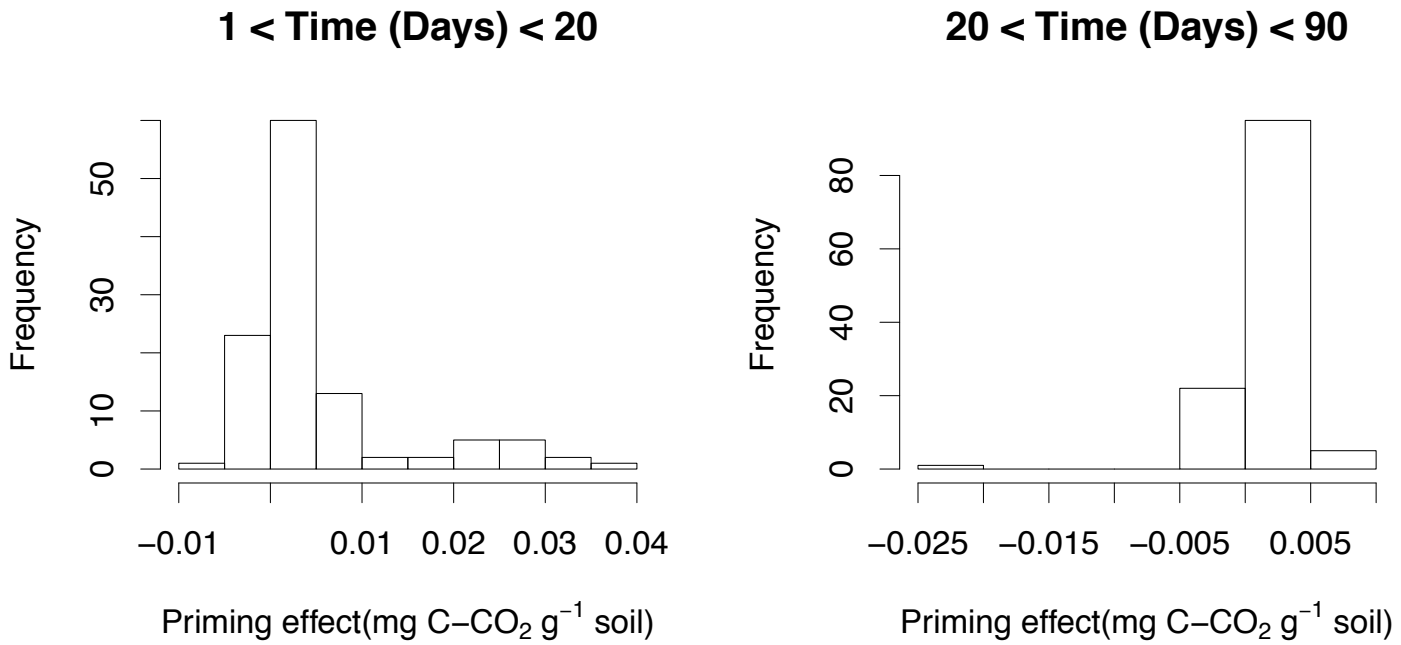
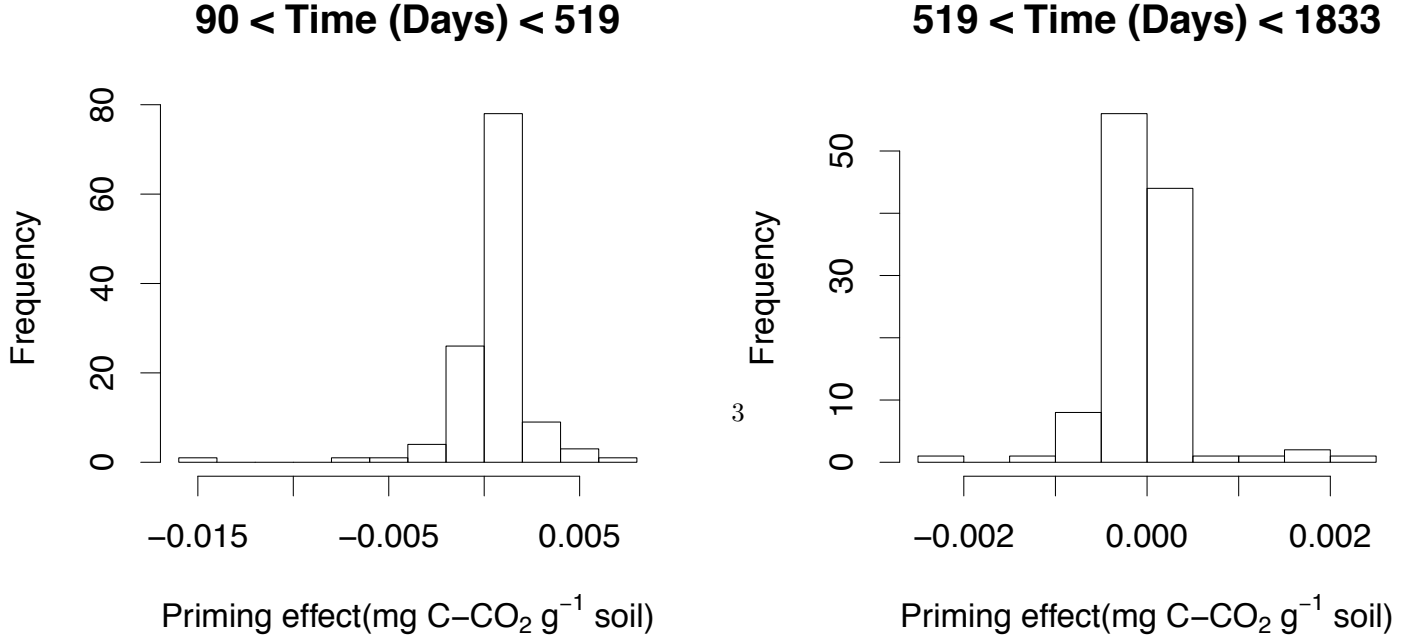
785

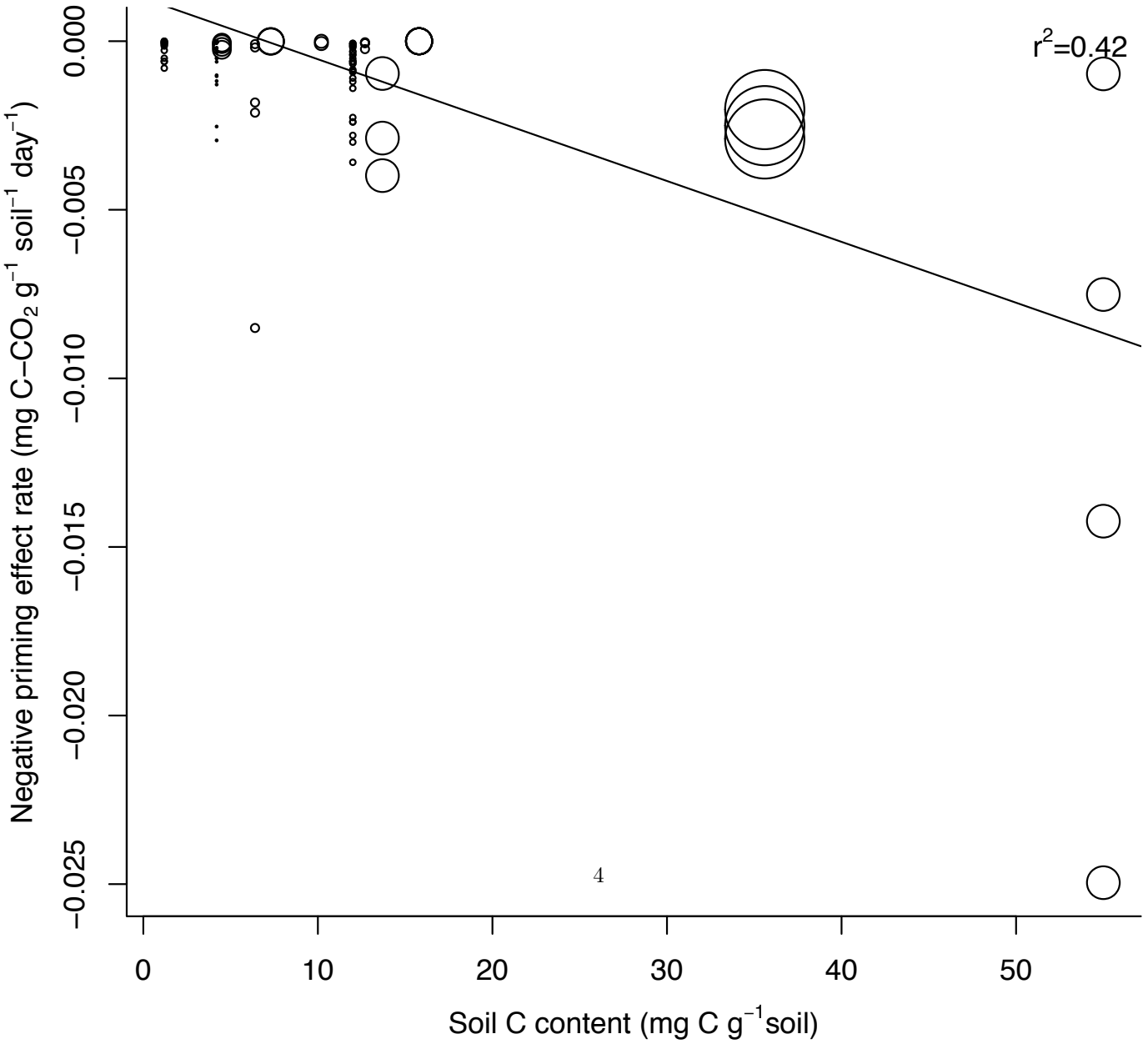
Figure captions

Figure 1: Priming effect as a function of time. The grey line represent the modelled rate of priming effect, while the black line represent the cumulative modelled priming effect, and is referred to the right y-axis. The size of the points is proportional to the weight of each case calculated using equation 1.
Figure 2: Frequency distribution of priming effect in the time quartiles of the database.
Figure 3: Negative priming effect as a function of soil C content.The size of the points is proportional to the weight of each case calculated using equation 1.


Priming effect rate (mg C–CO2 g−1 soil−1 day−1)
−0.02
0.00
0.02
0.04
0.06
0
200
400
600
800
1000
Time (days)
1 year
O2
−0.2
0.0
0.2
0.4
0.6
Cumulative priming effect (mg C–CO 2 g−1 soil−1)


1 < Time (Days) < 20
Frequency
0
10
30
50
−0.01
0.01
0.02
0.03
0.04
Priming effect(mg C−CO2 g−1 soil)
20 < Time (Days) < 90
Frequency
0
20
40
60
80
−0.025
−0.015
−0.005
0.005
Priming effect(mg C−CO2 g−1 soil)


90 < Time (Days) < 519
Frequency
0
20
40
60
80
−0.015
−0.005
0.005
Priming effect(mg C−CO2 g−1 soil)
519 < Time (Days) < 1833
Frequency
0
10
30
50
−0.002
0.000
0.002
Priming effect(mg C−CO2 g−1 soil)


Negative priming effect rate (mg C–CO2 g−1 soil−1 day−1)
0.000
−0.005
−0.010
−0.015
−0.020
−0.025
r2=0.42
0
10
20
30
40
50
Soil C content (mg C g−1soil)